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## Polyethyleneimine as tracer for electron microscopy

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## SUMMARY

In this thesis the development of a tracer particle for use in electron microscopy is described. Attempts were made to use this tracer particle in immuno-electron microscopy and to trace negatively charged tissue components.

After the introduction the requirements for a tracer particle for electron microscopy are discussed in chapter 2. A number of water-soluble synthetic polymers were theoretically and practically tested for their application as a tracer molecule. The most appropriate water-soluble polymer seemed to be poly-ethyleneimine (PEI).

In chapter 3 the applicability of PEI is described.

PEI is a polymerisation product of ethyleneimine and is commercially available in several molecular weights. Because of the presence of amino groups the polymer is highly soluble, but on the other hand strongly cationic. The molecule itself is not visible in the electron microscope but with the help of heavy metals it can be made electron dense. The reduction of  $\text{Ag}^+$  and  $\text{Au}^{3+}$  ions in a PEI solution gives rise to the formation of electron dense particles. In tissues PEI can be specifically stained with phosphotungstic acid (PTA). PEI can be detected down to a MW as low as 600. The advantage of PEI 600 is that it penetrates very rapidly in tissues and even in cell organelles. It can cause, however, tissue damage.

In chapter 4 the use of PEI 40.000 as an immuno tracer and as a tracer for anionic sites is described. Some antibodies were labeled with PEI 40.000 and subsequently tested. Although the first experiments were successful, it was impossible to use the obtained conjugates for IEM because of the strong nonspecific binding to tissues. This is probably caused by the strongly cationic character of PEI. On the other hand, the cationic character makes PEI very suitable for the tracing of anionic sites in tissues. Among others these sites were found along collagen fibrils and in basal laminae. Because of the regular distribution of PEI in these structures it is hypothesized that a relation exists between the composition of the two tissue components.

The anionic sites in the GBM especially are described in

chapter 5. Attention was paid to the distribution of the anionic sites in normal and abnormal situations. The distribution of the anionic sites in the GBM is most probably related to the filtration mechanism and to the formation of the GBM as well. The way in which the anionic sites influence the permeability of the GBM can be derived from the study on the presence and distribution of PEI in the GBM of rats with experimentally induced proteinuria. The findings that basement membrane collagen and fibrillar collagen are related to each other has resulted in the development of a GBM model as described in chapter 6. Apart from the findings, presented in this thesis, especially the data from Farquhar and co-workers concerning the glomerular filtration have also been used for the proposed model. The chemical information as to collagen was especially obtained from the group of Kefalides. The model fulfils the requirement of self-cleaning and contains possibilities for mechanical filtration, anionic charge and molecular sieving. It postulates a macromolecular filter consisting of a layer of procollagen molecules supplied with pores, being present on the epithelial cell surface. A widely meshed network is present in the lamina rara externa with glycosaminoglycans at the points of junction, giving rise to anionic sites. In the direction of the endothelium the width of the meshes of the network decreases and at that level (lamina rara interna) the main filtration barrier is situated.

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